

FORM PTO-1390 (Modified) (REV 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES		DESIGNATED/ELECTED OFFICE (DO/EO/US)		46342 /56776
CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
INTERNATIONAL APPLICATION NO. PCT/JP00/03720		INTERNATIONAL FILING DATE 8 June 2000		10 / 009693
				PRIORITY DATE CLAIMED 10 June 1999
TITLE OF INVENTION NOVEL PROTEIN AND DNA THEREOF				
APPLICANT(S) FOR DO/EO/US TAKEDA CHEMICAL INDUSTRIES, LTD.				
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:				
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409). 12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210). 				
Items 13 to 20 below concern document(s) or information included: <ol style="list-style-type: none"> 13. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 14. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 15. <input type="checkbox"/> A FIRST preliminary amendment. 16. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 17. <input type="checkbox"/> A substitute specification. 18. <input type="checkbox"/> A change of power of attorney and/or address letter. 19. <input checked="" type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail 23. <input checked="" type="checkbox"/> Other items or information: 				
PPCT/RO/101, 105; PCT/ISA/202, 2200, - PCT/IB301, 304, 308, 332 - PCT/IPEA/401, 402, 408, 416, Hard copy of Sequence Listing. Disk of Sequence Listing. Drawings: 2 sheets, 2 figures.				

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 10/009693	INTERNATIONAL APPLICATION NO. PCT/JP00/03720	ATTORNEY'S DOCKET NUMBER 46342 /56776
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24. The following fees are submitted.:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	\$1040.00
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$890.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$740.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	\$710.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	\$100.00

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$890.00

Surcharge of **\$130.00** for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).

\$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	37 - 20 =	17	x \$18.00	\$306.00
Independent claims	1 - 3 =	0	x \$84.00	\$0.00

Multiple Dependent Claims (check if applicable). **\$280.00**

TOTAL OF ABOVE CALCULATIONS = **\$1,606.00**

Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2. **\$0.00**

SUBTOTAL = **\$1,606.00**

Processing fee of **\$130.00** for furnishing the English translation later than 20 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). **\$0.00**

TOTAL NATIONAL FEE = **\$1,606.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). **\$40.00**

TOTAL FEES ENCLOSED = **\$1,646.00**

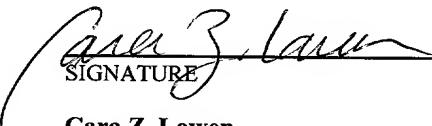
<input type="checkbox"/> Amount to be: refunded	\$
<input type="checkbox"/> charged	\$

- A check in the amount of **\$1,646.00** to cover the above fees is enclosed.
- Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.
- The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **04-1105** A duplicate copy of this sheet is enclosed.
- Fees are to be charged to a credit card. **WARNING: Information on this form may become public. Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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REGISTRATION NUMBER

10 December 2001

DATE

2/PST

1

SPECIFICATION

NOVEL PROTEIN AND ITS DNA

5

FIELD OF THE INVENTION

The present invention relates to a novel protein containing an amino acid sequence, which is the same or substantially the same as the amino acid sequence represented by SEQ ID NO:1 (preferably a protein having a GABA transporter activity; hereinafter sometimes referred to as the protein of the present invention) or its salts, or a DNA encoding the same.

BACKGROUND ART

15 γ -Aminobutyric acid (GABA), which is an inhibitory neurotransmitter, is released from GABAergic neurons and then removed from extracellular fluids by the action of GABA transporters located in neuronal terminals and glia cells. The mechanism of this active
20 uptake of GABA transporters is most important for determining when neurotransmission has been accomplished.

It is known that a GABA transporter has four subtypes (GAT-1, GAT-2, GAT-3 and BGT-1). GAT-1 and
25 GAT-3 are located in brain and retina, GAT-2 in almost all organs, and BGT-1 in kidney and brain. The GAT-1 gene was cloned from mouse (Gene Bank accession No. M92378), rat (Gene Bank accession No. M33003) and human (Gene Bank accession No. X54673); the GAT-2 gene from
30 mouse (Gene Bank accession No. L04663) and rat (Gene Bank accession No. M95762); the GAT-3 gene from mouse (Gene Bank accession No. L04662) and rat (Gene Bank accession No. M95763); and the BGT-1 gene from mouse (Gene Bank accession No. M97632) and dog (Gene Bank

accession No. M80403). However, a human GAT-2 gene is yet unknown so far.

It is an object of the present invention to provide the following:

- 5 (i) a protein containing an amino acid sequence, which is the same or substantially the same as the amino acid sequence represented by SEQ ID NO:1 (preferably, a protein having a GABA transporter activity) or its partial peptide, or its amide or ester, or a salt thereof;
- 10 (ii) a DNA encoding the protein containing an amino acid sequence, which is the same or substantially the same as the amino acid sequence represented by SEQ ID NO:1 (preferably, a protein having a GABA transporter activity) or its partial peptide;
- 15 (iii) a recombinant vector containing the DNA;
- (iv) a transformant bearing the recombinant vector;
- 20 (v) a process of producing the protein containing an amino acid sequence, which is the same or substantially the same as the amino acid sequence represented by SEQ ID NO:1 (preferably, a protein having a GABA transporter activity) or a salt thereof;
- 25 (vi) an antibody to the protein containing an amino acid sequence, which is the same or substantially the same as the amino acid sequence represented by SEQ ID NO:1 (preferably, a protein having a GABA transporter activity) or its partial peptide, or its amide or ester, or a salt thereof;
- 30 (vii) a method of screening a compound that alters a GABA transporter activity of the protein containing an amino acid sequence, which is the same or substantially the same as the amino acid sequence represented by SEQ ID NO:1 (preferably, a protein having a GABA transporter activity), or its salt;
- 35

(viii) a compound or its salt obtainable by the screening method; and,

(ix) a pharmaceutical comprising the compound or its salt.

5

DISCLOSURE OF THE INVENTION

The present inventors have made extensive studies and as a result, succeeded in isolating cDNA encoding human-derived GABA transporter protein having a high homology to mouse- or rat-derived GAT-2, sequencing the entire base sequence and then expressing cDNA in cells. Based on these findings, the present inventors have come to accomplish the present invention.

That is, the present invention provides the following:

(1) A protein containing an amino acid sequence, which is the same or substantially the same amino acid sequence represented by SEQ ID NO:1, or its salt.

(2) The protein or its salt according to (1), which has a GABA transporter activity.

(3) A partial peptide of the protein according to (1), its amide or ester, or a salt thereof.

(4) A DNA containing a DNA having a base sequence encoding the protein according to (1) or the partial peptide according to (3).

(5) The DNA according to (4) having a base sequence represented by SEQ ID NO:2.

(6) A recombinant vector containing the DNA according to (4).

(7) A transformant transformed with the recombinant vector according to (6).

(8) A method of producing the protein or its salt, according to (1) or the partial peptide, its amide or ester, or a salt thereof, according to (3), which comprises culturing said transformant according to (7),

producing and accumulating the protein according to (1) or the partial peptide according to (3), and collecting the protein or partial peptide.

5 (9) An antibody to the protein or its salt according to (1) or to the partial peptide, its amide or ester, or a salt thereof according to (3).

10 (10) A method of screening a compound that promotes or inhibits a GABA transporter activity, which comprises assaying the GABA transporter activity using the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof according to (3).

15 (11) A method for screening a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3), which comprises using the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3).

20 (12) A kit for screening a compound or its salt that promotes or inhibits a GABA transporter activity, comprising the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3).

25 (13) A kit for screening a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3), comprising the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3).

30 (14) A compound or its salt that promotes or inhibits a GABA transporter activity, obtainable using the screening method according to (10) or using the screening kit according to (12).

(15) A compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3), 5 which is obtainable using the screening method according to (11) or using the screening kit according to (13).

(16) A pharmaceutical product comprising a compound or its salt that promotes or inhibits a GABA 10 transporter activity, which is obtainable using the screening method according to (10) or using the screening kit according to (12).

(17) A pharmaceutical product comprising a compound or its salt that promotes or inhibits a GABA 15 transporter activity of the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3), which is obtainable using the screening method according to (11) or using the screening kit according to (13).

(18) A pharmaceutical product comprising a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof, according to (3), which is a 25 medicament for the prevention/treatment of anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic spinal paralysis, spondylosis deformans, spinocerebellar degeneration, spastic paralysis accompanied by multiple sclerosis, 30 tension headache, lumbago, and neck, shoulder and arm syndrome.

(19) A pharmaceutical product comprising a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt 35 according to (1), or the partial peptide, its amide or

ester, or a salt thereof, according to (3), which is a medicament for the prevention/treatment of dementia.

More specifically, the present invention provides the following:

5 (20) The protein or its salt according to (1), wherein the protein is a protein or its salt containing (i) the amino acid sequence represented by SEQ ID NO:1, an amino acid sequence represented by SEQ ID NO:1, wherein at least 1 or 2 (preferably approximately 1 to 10, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are deleted; (ii) an amino acid sequence represented by SEQ ID NO:1, to which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 10 and most preferably several (1 or 2)) amino acids are added; (iii) an amino acid sequence represented by SEQ ID NO:1, in which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 10 and most preferably several (1 or 2)) amino acids are substituted with other amino acids; and (iv) a combination of the above amino acid sequences.

20 (21) A method of screening according to (10) or (11), which comprises comparing the case wherein a test compound is contacted with the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof according to (3), and the case wherein the test compound is not contacted with the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof according to (3).

25 (22) A method of screening a compound or its salt that alters a GABA transporter activity, which comprises comparing the case wherein a test compound is contacted with a cell containing the protein according to (1), and the case wherein the test compound is not

contacted with the cell containing the protein according to (1).

(23) A method of screening a compound or its salt that alters a GABA transporter activity of the protein according to (1), which comprises comparing the case wherein a test compound is contacted with the protein according to (1) expressed on a cell membrane of the transformant according to (7) by incubation of the transformant, and the case wherein the test compound is not contacted with the protein according to (1) expressed on the cell membrane of the transformant according to (7) by incubation of the transformant.

(24) A compound or its salt that alters a GABA transporter activity of the protein according to (1), which is obtainable by the screening method according to (21) through (23).

(25) A composition for the prevention/treatment of anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic spinal paralysis, spondylosis deformans, spinocerebellar degeneration, spastic paralysis accompanied by multiple sclerosis, tension headache, lumbago, and neck, shoulder and arm syndrome, comprising a compound or its salt that alters the function of the protein according to (1), which is obtainable by the screening method according to (21) through (23).

(26) A method for quantification of the protein or its salt according to (1), or the partial peptide, its amide or ester, or a salt thereof according to (3) in a test sample fluid, which comprises contacting the antibody according to (9) with the test sample fluid.

(27) A composition for the prevention/treatment of anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic

spinal paralysis, spondylosis deformans,
spinocerebellar degeneration, spastic paralysis
accompanied by multiple sclerosis, tension headache,
lumbago, and neck, shoulder and arm syndrome,
5 comprising the antibody according to (9).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows hydrophobic plotting prepared based
on the amino acid sequence of the human-derived protein
10 of the present invention, obtained in EXAMPLES.

FIG. 2 shows the results of increased amounts of
labeled GABA uptake assayed after adding [³H]-GABA
to hGAT2/LM(TK⁻) cells where the protein of the
present invention was expressed, in a buffer
15 containing sodium ions and chlorine ions. In the left
graph, hGAT2/LM(TK⁻) designates the LM(TK⁻) cell
wherein the human-derived protein of the present
invention was expressed, and in the right graph,
Mock/LM(TK⁻) designates the LM(TK⁻) cell inserted
20 with plasmid pMCMVneo. The numerical value on the
left side denotes the assay results of amounts of
labeled GABA uptake measured after adding 50 nM
[³H]-GABA to hGAT2/LM(TK⁻) cells where the protein
of the present invention was expressed, in a
25 buffer containing sodium ions and chlorine ions. The
data is shown as a mean value ± standard error.

BEST MODE FOR CARRYING OUT THE INVENTION

The protein of the present invention is a protein
30 which has the same or substantially the same as the
amino acid sequence shown by SEQ ID NO:1.

The protein of the present invention may be any
protein derived from any cells of human and other warm-
blooded animals (e.g., guinea pig, rat, mouse, rabbit,
35 swine, sheep, bovine, monkey, etc.) such as splenocyte,

nerve cells, glial cells, β cells of pancreas, bone marrow cells, mesangial cells, Langerhans' cells, epidermic cells, epithelial cells, endothelial cells, fibroblasts, fibrocytes, myocytes, fat cells, immune 5 cells (e.g., macrophage, T cells, B cells, natural killer cells, mast cells, neutrophils, basophils, eosinophils, monocytes), megakaryocytes, synovial cells, chondrocytes, bone cells, osteoblasts, osteoclasts, mammary gland cells, hepatocyte or interstitial cell; 10 or the corresponding precursor cells, stem cells, cancer cells, etc.; or any tissues where such cells are present, such as brain or any of brain regions (e.g., olfactory bulb, amygdaloid nucleus, basal ganglia, hippocampus, thalamus, hypothalamus, subthalamic 15 nucleus, cerebral cortex, medulla oblongata, cerebellum, occipital lobes, frontal lobe, lateral lobe, putamen, caudate nucleus, corpus callosum, substantia nigra), spinal cord, hypophysis, stomach, pancreas, kidney, liver, gonad, thyroid, gall-bladder, bone marrow, 20 adrenal gland, skin, muscle, lung, gastrointestinal tract (e.g., large intestine and small intestine), blood vessel, heart, thymus, spleen, submandibular gland, peripheral blood, peripheral hemocytes, prostate, testis, ovary, placenta, uterus, bone, joint, skeletal 25 muscle, etc. (especially, brain or any of brain regions); the proteins may also be synthetic proteins.

The amino acid sequence which is substantially the same as the amino acid sequence represented by SEQ ID NO:1 includes, for example, an amino acid sequence 30 having at least about 95% homology, preferably at least about 98% homology, to the amino acid sequence represented by SEQ ID NO:1.

Examples of the protein containing the amino acid sequence which is substantially the same as the amino 35 acid sequence represented by SEQ ID NO:1 include a

protein containing the amino acid sequence which is substantially the same as the amino acid sequence represented by SEQ ID NO:1 and has an activity substantially equivalent to that of the amino acid sequence represented by SEQ ID NO:1, etc.

The term "substantially equivalent" is used to mean that the activities of the protein, for example, a transporter activity (GABA transporter activity), physiological properties, etc. are substantially equivalent. Substitution, deletion, addition or insertion of an amino acid(s) do not often cause significant changes in physiological or chemical properties of polypeptides; in such a case, proteins that have undergone such substitution, deletion, addition or insertion will be considered to be substantially equivalent to intact proteins that do not receive undergo the substitution, deletion, addition or insertion. Substantially the same substituent(s) of an amino acid(s) in the amino acid sequence can be selected from, e.g., other amino acids of the class to which the amino acid(s) belong. Examples of non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, methionine and the like. Examples of polar (neutral) amino acids are glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, and the like. Examples of positively charged (basic) amino acids are arginine, lysine, histidine, and the like. Examples of negatively charged (acidic) amino acids include aspartic acid, glutamic acid, and the like.

The substantially equivalent activities are used to mean that these activities are equivalent in terms of its nature (physiologically or pharmacologically). Therefore, it is preferred that these activities are equivalent to each other, but it is allowable that

differences among grades such as the level of these activities and molecular weight of the protein may be present.

5 The transporter activity can be determined according to a publicly known method, for example, by means of screening which will be later described.

As the protein of the present invention, there may be employed proteins containing (i) the amino acid sequence represented by SEQ ID NO:1, of which at least 10 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are deleted; (ii) the amino acid sequence represented by SEQ ID NO:1, to which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are added; (iii) the amino acid sequence represented by SEQ ID NO:1, in which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are substituted with other amino acids; and (iv) a combination of the above amino acid sequences. Furthermore, examples of the proteins of the present invention include variants of the proteins described 25 above, wherein the amino group at the N-terminal methionine residue of the proteins is protected with a protecting group (e.g., a C₁₋₆ acyl group such as a C₂₋₆ alkanoyl group, e.g., formyl group, acetyl group, etc.); those wherein the N-terminal region is cleaved 30 in vivo and Gln thus formed is pyroglutaminated; those wherein a substituent (e.g., -OH, -SH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain of an amino acid in the molecule is protected with a suitable protecting group (e.g., a C₁₋₆ acyl group such as a C₂₋₆ alkanoyl group, e.g., formyl 35

group, acetyl group, etc.), or conjugated proteins such as glycoproteins having sugar chains.

The partial peptides of the proteins of the present invention (hereinafter sometimes merely referred to as the partial peptides) may be any partial peptides so long as they are partial peptides of the proteins of the present invention described above, but exposed extracellularly sites, etc. in the proteins of the present invention may be used.

Specifically, the partial peptides of the proteins containing the amino acid sequence represented by SEQ ID NO:1 are peptides containing the region determined to be an extracellular region (hydrophilic site) in the hydrophilic plotting shown in [FIG. 1]. Peptides containing in part the hydrophobic site may also be employed similarly. Peptides containing respective domains separately may also be employed, and peptides containing the site bearing a plurality of the domains at the same time may be used as well.

Also the partial peptides of the present invention, may be those containing the amino acid sequences described above, of which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are deleted; to which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are added; or, in which at least 1 or 2 (preferably approximately 1 to 30, more preferably approximately 1 to 9 and most preferably several (1 or 2)) amino acids are substituted with other amino acids.

In the partial proteins of the present invention, the C-terminus is usually in the form of a carboxyl group (-COOH) or a carboxylate (-COO⁻) but may be in

the form of an amide (-CONH₂) or an ester (-COOR) (wherein R has the same significance as defined above).

5 The partial peptides of the present invention further include those containing substituents on the side chains of intramolecular amino acids, which are protected with appropriate protecting groups, conjugated peptides such as so-called glycoproteins having sugar chains bound thereto, and the like.

10 The partial peptide of the present invention can be employed as an antigen for producing an antibody and therefore, does not necessarily possess the GABA transporter activity.

15 As salts of the protein of the present invention or its partial peptide, physiologically acceptable acid addition salts are particularly preferred. Examples of such salts employed are salts with, e.g., inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), or salts with organic acids (e.g., acetic acid, formic acid, propionic acid, 20 fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

25 The protein of the present invention or its salts may be manufactured by a publicly known purification method from human or the other warm-blooded animal cells or tissues described above. Alternatively, the protein of the present invention or its salts may also be manufactured by culturing a transformant containing 30 DNA encoding the protein of the present invention, which will be later described. Furthermore, the protein of the present invention or its salts may also be manufactured by the methods for synthesizing proteins, which will also be described hereinafter, or by a 35 modification of such methods.

Where the protein or its salts are manufactured from human or mammalian tissues or cells, the human or mammalian tissues or cells are homogenized, then extracted with an acid or the like, and the extract is 5 isolated and purified by a combination of chromatography techniques such as reverse phase chromatography, ion exchange chromatography, and the like.

The partial peptide of the protein of the present 10 invention or its salts can be manufactured by publicly known methods for peptide synthesis, or by cleaving the protein of the present invention with an appropriate peptidase. For the methods for peptide synthesis, for example, either solid phase synthesis or liquid phase 15 synthesis may be used. That is, the partial peptide or amino acids that can construct the protein of the present invention are condensed with the remaining part of the partial peptide of the present invention. Where the product contains protecting groups, these 20 protecting groups are removed to give the desired peptide. Publicly known methods for condensation and elimination of the protecting groups are described in 1) - 5) below.

25 1) M. Bodanszky & M.A. Ondetti: *Peptide Synthesis*,
Interscience Publishers, New York (1966)

2) Schroeder & Luebke: *The Peptide*, Academic Press,
New York (1965)

3) Nobuo Izumiya, et al.: *Peptide Gosei-no-Kiso to*
30 *Jikken* (Basics and experiments of peptide synthesis),
published by Maruzen Co. (1975)

4) Haruaki Yajima & Shunpei Sakakibara: *Seikagaku*
Jikken Koza (Biochemical Experiment) 1, *Tanpakushitsu*
no Kagaku (Chemistry of proteins) IV, 205 (1977)

5) Haruaki Yajima ed.: *Zoku Iyakuhin no Kaihatsu*
(A sequel to Development of Pharmaceuticals), Vol. 14,
Peptide Synthesis, published by Hirokawa Shoten

5 After completion of the reaction, the product may
be purified and isolated by a combination of
conventional purification methods such as solvent
extraction, distillation, column chromatography, liquid
chromatography and recrystallization to give the
10 partial peptide of the present invention. When the
partial peptide obtained by the above methods is in a
free form, the peptide can be converted into an
appropriate salt by a publicly known method or a
modification of the known method; conversely when the
15 protein is obtained in a salt form, it can be converted
into a free form or a different salt form by a publicly
known method or a modification of the known method.

The DNA encoding the protein of the present
invention may be any DNA so long as it contains the
20 base sequence encoding the protein of the present
invention described above. Such a DNA may also be any
one of genomic DNA, genomic DNA library, cDNA derived
from the cells or tissues described above, cDNA library
derived from the cells or tissues described above and
25 synthetic DNA. The vector to be used for the library
may be any of bacteriophage, plasmid, cosmid, phagemid
and the like. In addition, the DNA can be directly
amplified by reverse transcriptase polymerase chain
reaction (hereinafter abbreviated as RT-PCR) with total
30 RNA or mRNA fraction prepared from the above-described
cells or tissues.

Specifically, the DNA encoding the protein of the
present invention may be any one of, for example, DNA
containing the base sequence represented by SEQ ID NO:2,
35 or any DNA having a base sequence hybridizable to the

base sequence represented by SEQ ID NO:2 under high stringent conditions and encoding a protein which has the activities substantially equivalent to those of the protein of the present invention (e.g., a GABA transporter activity, etc.).

Specific examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO:2 include DNA having at least about 90% homology, preferably at least about 95% homology, and more preferably at least about 98% homology, to the base sequence represented by SEQ ID NO:2.

The hybridization can be carried out by publicly known methods or by a modification thereof, for example, according to the method described in Molecular Cloning, 15 2nd Ed., J. Sambrook et al., Cold Spring Harbor Lab. Press, (1989). A commercially available library may also be used according to the instructions of the attached manufacturer's protocol. Preferably, the hybridization can be carried out under high stringent 20 conditions.

The high stringent conditions used herein are, for example, those in a sodium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM at a temperature of about 50°C to about 70°C, 25 preferably about 60°C to about 65°C. In particular, hybridization conditions in a sodium concentration at about 19 mM at a temperature of about 65°C are most preferred.

More specifically, for the DNA encoding the 30 protein having the amino acid sequence represented by SEQ ID NO:1, there may be employed DNA having the base sequence represented by SEQ ID NO:2, etc.

The DNA encoding the partial peptide of the present invention may be any DNA so long as it contains 35 the base sequence encoding the partial peptide of the

present invention described above. The DNA may also be any of genomic DNA, genomic DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described 5 above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the like. In addition, the DNA can be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) with 10 total RNA or mRNA fraction prepared from the above-described cells or tissues.

Specifically, the DNA encoding the partial peptide of the present invention includes, for example, DNA containing a partial base sequence of the DNA having 15 the base sequence represented by SEQ ID NO:2, or (ii) DNA which has a base sequence hybridizable to the base sequence represented by SEQ ID NO:2 under high stringent conditions and contains a partial base sequence of the DNA encoding the protein having the 20 activities substantially equivalent to those of the protein of the present invention (e.g., a GABA transporter activity, etc.).

Specific examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO:2 include 25 DNA having at least about 95% homology, preferably at least about 98% homology, to the base sequence represented by SEQ ID NO:2. The hybridization and the high stringent conditions used are those as described above.

30 For cloning of the DNA that completely encodes the protein or its partial peptide of the present invention (hereinafter collectively referred to as the protein of the present invention), (1) the DNA may be amplified by PCR using synthetic DNA primers containing a part of 35 the base sequence of the protein of the present

invention, or (2) the DNA inserted into an appropriate vector can be selected by hybridization with a labeled DNA fragment or synthetic DNA that encodes a part or the entire region of the protein of the present
5 invention, etc. The hybridization can be carried out, for example, according to the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989) or the like. The hybridization may also be performed using commercially available
10 library in accordance with the protocol described in the attached instructions.

Conversion (deletion, addition or substitution) of the base sequence for DNA can be effected by publicly known methods such as the Gapped duplex method or the
15 Kunkel method or modifications thereof, using a publicly known kit available as MutanTM-G or MutanTM-K (both Takara Shuzo Co., Ltd.).

The cloned DNA encoding the protein of the present invention can be used as it is, or, if desired, after
20 digestion with restriction enzymes or after addition of linkers thereto, depending upon purposes. The DNA may contain ATG as a translation initiation codon at the 5' end thereof and TAA, TGA or TAG as a translation termination codon at the 3' end thereof. These
25 translation initiation and termination codons may also be added by using an appropriate synthetic DNA adapter.

The expression vector of the protein of the present invention can be manufactured, for example, by
30 (a) excising the desired DNA fragment from the DNA encoding the protein of the present invention, (b) and then ligating the DNA fragment with an appropriate expression vector downstream a promoter in the expression vector.

Examples of the vector include plasmids derived
35 from E. coli (e.g., pBR322, pBR325, pUC12, pUC13),

plasmids derived from *Bacillus subtilis* (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19, pSH15), bacteriophages such as λ phage, etc., animal viruses such as retrovirus, vaccinia virus, baculovirus, 5 etc. as well as pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNA1/Neo, etc.

The promoter used in the present invention may be any promoter if it matches well with a host to be used for gene expression. In the case of using animal cells 10 as the host, examples of the promoter include SR α promoter, SV40 promoter, HIV-LTR promoter, CMV promoter, HSV-TK promoter, etc. Among them, CMV promoter or SR α promoter is preferably used. Where the host is bacteria belonging to the genus *Escherichia*, preferred examples 15 are trp promoter, lac promoter, recA promoter, λ PL promoter, lpp promoter, etc. In the case of using bacteria of the genus *Bacillus* as the host, preferred example are SPO1 promoter, SPO2 promoter, penP promoter, etc. When yeast is used as the host, preferred examples 20 are PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, etc. When insect cells are used as the host, preferred examples are polyhedrin promoter, P10 promoter, etc.

In addition to the foregoing examples, the 25 expression vector may further optionally contain an enhancer, a splicing signal, a poly A addition signal, a selection marker, SV40 replication origin (hereinafter sometimes abbreviated as SV40ori), etc. Examples of the selection marker include dihydrofolate 30 reductase (hereinafter sometimes abbreviated as dhfr) gene [methotrexate (MTX) resistance], ampicillin resistant gene (hereinafter sometimes abbreviated as Amp^r), neomycin resistant gene (hereinafter sometimes abbreviated as Neo, G418 resistance), etc. If necessary, 35 a signal sequence that matches with a host is added to

the N-terminus of the protein of the present invention. Examples of the signal sequence that can be used are PhoA signal sequence, OmpA signal sequence, etc. in the case of using bacteria of the genus Escherichia as the host; α -amylase signal sequence, subtilisin signal sequence, etc. in the case of using bacteria of the genus Bacillus as the host; MFA signal sequence, SUC2 signal sequence, etc. in the case of using yeast as the host; and insulin signal sequence, α -interferon signal sequence, antibody molecule signal sequence, etc. in the case of using animal cells as the host, respectively.

Using the vector containing the DNA encoding the protein of the present invention thus constructed, transformants can be manufactured.

Examples of the host, which may be employed, are bacteria belonging to the genus Escherichia, bacteria belonging to the genus Bacillus, yeast, insect cells, insects and animal cells, etc.

Specific examples of the bacteria belonging to the genus Escherichia include Escherichia coli K12 DH1 (Proc. Natl. Acad. Sci. U.S.A., 60, 160 (1968)), JM103 (Nucleic Acids Research, 9, 309 (1981)), JA221 (Journal of Molecular Biology, 120, 517 (1978)), HB101 (Journal of Molecular Biology, 41, 459 (1969)), C600 (Genetics, 39, 440 (1954)), etc.

Examples of the bacteria belonging to the genus Bacillus include Bacillus subtilis MI114 (Gene, 24, 255 (1983)), 207-21 (Journal of Biochemistry, 95, 87 (1984)), etc.

Examples of yeast employed are *Saccharomyces cerevisiae* AH22, AH22R, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, *Pichia pastoris* KM71, etc.

As insect cells, there may be employed, *Spodoptera frugiperda* cells (Sf cells), MG1 cells derived from mid-intestine of *Trichoplusia ni*, High Five™ cells derived from egg of *Trichoplusia ni*, cells derived from 5 *Mamestra brassicae*, cells derived from *Estigmene acrea*, etc., when the virus is AcNPV; when the virus is BmNPV, *Bombyx mori* N cells (BmN cells), etc. are used. Examples of the Sf cells which can be used are Sf9 cells (ATCC CRL1711) and Sf21 cells (both cells are 10 described in Vaughn, J. L. et al., *In Vivo*, 13, 213-217 (1977)).

As the insect, for example, a larva of *Bombyx mori* can be used (Maeda et al., *Nature*, 315, 592 (1985)).

Examples of animal cells include monkey cell COS-7, 15 Vero, Chinese hamster cell CHO (hereinafter abbreviated as CHO cells), dhfr gene deficient Chinese hamster cell CHO (hereinafter abbreviated as CHO (dhfr') cells), mouse L cells, mouse AtT-20, mouse myeloma cells, rat GH 3, human FL cells, HEK293 cells, C127 cells, BALB3T3 20 cells, Sp-2 cells, etc. Among them, CHO cells, CHO (dhfr') cells, HEK293 cells, LM cells, etc. are preferred.

Bacteria belonging to the genus *Escherichia* can be transformed, for example, by the method described in 25 Proc. Natl. Acad. Sci. U.S.A., 69, 2110 (1972), Gene, 17, 107 (1982), etc.

Bacteria belonging to the genus *Bacillus* can be transformed, for example, by the method described in Molecular & General Genetics, 168, 111 (1979).

30 Yeast can be transformed, for example, by the method described in Methods in Enzymology, 194, 182-187 (1991), Proc. Natl. Acad. Sci. U.S.A., 75, 1929 (1978), etc.

Insect cells or insects can be transformed, for example, according to the method described in Bio/Technology, 6, 47-55 (1988).

Methods for introducing the expression vectors 5 into animal cells include, for example, the calcium phosphate method [Graham, F. L. and van der Eb, A., J. Virology, 52, 456-467 (1973)], the electroporation method [Nuemann, E. et al., EMBO J., 1, 841-845 (1982)], etc.

10 As described above, transformants transformed with the expression vector containing the DNA encoding the protein of the present invention can be obtained.

For stably expressing the protein of the present invention using animal cells, there is applicable a 15 method of selecting the cells by clone selection in which the aforesaid expression vectors transfected to animal cells are introduced into chromosomes.

Specifically, transformants are selected using as an index the selection marker described above. Further by 20 repeated clone selections on the transformants thus obtained using the selection marker, stable animal cell line capable of highly expressing the protein of the present invention can be obtained. Furthermore, when the dhfr gene is used as a selection marker, 25 cultivation can be performed by gradually increasing a level of MTX, resistant cells are selected thereby to amplify the DNA encoding the protein of the present invention in the cells together with the dhfr gene. Thus, the animal cell line of higher expression can be 30 obtained.

The transformant described above is cultivated under conditions that the DNA encoding the protein of the present invention can express, to produce and accumulate the protein of the present invention. Thus,

the protein of the present invention or its salts can be produced.

Where the host is bacteria belonging to the genus Escherichia or the genus Bacillus, the transformant can 5 be appropriately incubated in a liquid medium, which contains materials required for growth of the transformant such as carbon sources, nitrogen sources, inorganic materials, etc. Examples of the carbon sources include glucose, dextrin, soluble starch, 10 sucrose, etc. Examples of the nitrogen sources include inorganic or organic materials such as ammonium salts, nitrate salts, corn steep liquor, peptone, casein, meat extract, soybean cake, potato extract, etc. Examples of the inorganic materials are calcium chloride, sodium 15 dihydrogenphosphate, magnesium chloride, etc. In addition, yeast extract, vitamins, growth promoting factors, etc. may also be added to the medium. Preferably, pH of the medium is adjusted to about 5 to about 8.

20 A preferred example of the medium for incubation of the bacteria belonging to the genus Escherichia is M9 medium supplemented with glucose and Casamino acids (Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972). 25 If necessary, a chemical such as 3β -indolylacrylic acid can be added to the medium thereby to activate the promoter efficiently.

Where the bacteria belonging to the genus Escherichia are used as the host, the transformant is 30 usually cultivated at about 15°C to about 43°C for about 3 hours to about 24 hours. If necessary, the culture may be aerated or agitated.

Where the bacteria belonging to the genus Bacillus are used as the host, the transformant is cultivated 35 generally at about 30°C to about 40°C for about 6 hours

to about 24 hours. If necessary, the culture can be aerated or agitated.

Where yeast is used as the host, the transformant is cultivated, for example, in Burkholder's minimal medium (Bostian, K. L. et al., Proc. Natl. Acad. Sci. U.S.A., 77, 4505 (1980)) or in SD medium supplemented with 0.5% Casamino acids (Bitter, G. A. et al., Proc. Natl. Acad. Sci. U.S.A., 81, 5330 (1984)). Preferably, pH of the medium is adjusted to about 5 to about 8. In general, the transformant is cultivated at about 20°C to about 35°C for about 24 hours to about 72 hours. If necessary, the culture can be aerated or agitated.

Where insect cells or insects are used as the host, the transformant is cultivated in, for example, Grace's Insect Medium (Grace, T. C. C., Nature, 195, 788 (1962)) to which an appropriate additive such as immobilized 10% bovine serum is added. Preferably, pH of the medium is adjusted to about 6.2 to about 6.4. Normally, the transformant is cultivated at about 27°C for about 3 days to about 5 days and, if necessary, the culture can be aerated or agitated.

Where animal cells are employed as the host, the transformant is cultivated in, for example, MEM medium containing about 5% to about 20% fetal bovine serum (Science, 122, 501 (1952)), DMEM medium (Virology, 8, 396 (1959)), RPMI 1640 medium (The Journal of the American Medical Association, 199, 519 (1967)), 199 medium (Proceeding of the Society for the Biological Medicine, 73, 1 (1950)), etc. Preferably, pH of the medium is adjusted to about 6 to about 8. The transformant is usually cultivated at about 30°C to about 40°C for about 15 hours to about 72 hours and, if necessary, the culture can be aerated or agitated.

As described above, the protein of the present invention can be produced in the cell membrane of the transformant, etc.

5 The protein of the present invention can be separated and purified from the culture described above by the following procedures.

When the protein of the present invention is extracted from the culture or cells, after cultivation the transformant or cell is collected by a publicly 10 known method and suspended in an appropriate buffer. The transformant or cell is then disrupted by publicly known methods such as ultrasonication, a treatment with lysozyme and/or freeze-thaw cycling, followed by centrifugation, filtration, etc. Thus, the crude 15 extract of the protein of the present invention can be obtained. The buffer used for the procedures may contain a protein modifier such as urea or guanidine hydrochloride, or a surfactant such as Triton X-100TM, etc.

20 The protein of the present invention contained in the extract thus obtained can be purified by appropriately combining the publicly known methods for separation and purification. Such publicly known methods for separation and purification include a 25 method utilizing difference in solubility such as salting out, solvent precipitation, etc.; a method mainly utilizing difference in molecular weight such as dialysis, ultrafiltration, gel filtration, SDS-polyacrylamide gel electrophoresis, etc.; a method 30 utilizing difference in electric charges such as ion exchange chromatography, etc.; a method utilizing difference in specific affinity such as affinity chromatography, etc.; a method utilizing difference in hydrophobicity such as reverse phase high performance 35 liquid chromatography, etc.; a method utilizing

difference in isoelectric point such as isoelectrofocusing electrophoresis; and the like.

When the protein of the present invention thus obtained is in a free form, it can be converted into 5 the salt by publicly known methods or modifications thereof. On the other hand, when the protein is obtained in the form of a salt, it can be converted into the free form or in the form of a different salt by publicly known methods or modifications thereof.

10 The protein of the present invention produced by the recombinant can be treated, prior to or after the purification, with an appropriate protein modifying enzyme so that the protein can be appropriately modified to partially remove a polypeptide. Examples of 15 the protein-modifying enzyme include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase and the like.

20 The thus produced protein of the present invention or salts thereof can be assayed by an enzyme immunoassay using a specific antibody, or the like.

Antibodies to the protein of the present invention, 25 its partial peptide, or salts thereof may be any of polyclonal antibodies and monoclonal antibodies, as long as they are capable of recognizing the protein of the present invention, its partial peptide, or salts thereof.

The antibodies to the protein of the present invention, its partial peptide, or salts thereof (hereinafter merely referred to as the protein of the 30 present invention) may be manufactured by publicly known methods for manufacturing antibodies or antisera, using as antigens the protein of the present invention. [Preparation of monoclonal antibody]

(a) Preparation of monoclonal antibody-producing cells

The protein of the present invention is administered to mammal either solely or together with carriers or diluents to the site where the production of antibody is possible by the administration. In order

5 to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every two to six weeks and about 2 to about 10 times in total.

10 Examples of the applicable mammals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep and goats, with the use of mice and rats being preferred.

In the preparation of monoclonal antibody-producing cells, a warm-blooded animal, e.g., mice, 15 immunized with an antigen wherein the antibody titer is noted is selected, then spleen or lymph node is collected after two to five days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells from homozoic or 20 heterozoic animal to give monoclonal antibody-producing hybridomas. Assay of the antibody titer in antisera may be carried out, for example, by reacting a labeled form of the protein of the present invention, which will be described later, with the antiserum followed by 25 assaying the binding activity of the labeling agent bound to the antibody. The fusion may be carried out, for example, through the known method by Kohler and Milstein (Nature, 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), 30 Sendai virus, etc., of which PEG is preferably employed.

Examples of the myeloma cells are those collected from warm-blooded animals such as NS-1, P3U1, SP2/0, AP-a, etc. In particular, P3U1 is preferably employed. A preferred ratio of the count of the antibody-producing cells used (spleen cells) to the count of

myeloma cells is within a range of approximately 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of approximately 10 to 80% followed by incubating at about 20 to about 40°C, 5 preferably at about 30 to about 37°C for about 1 to about 10 minutes, an efficient cell fusion can be carried out.

Various methods can be used for screening of a 10 monoclonal antibody-producing hybridoma. Examples of such methods include a method which comprises adding the supernatant of hybridoma to a solid phase (e.g., microplate) adsorbed with the protein of the present invention as an antigen directly or together with a carrier, adding an anti-immunoglobulin antibody (where 15 mouse cells are used for the cell fusion, anti-mouse immunoglobulin antibody is used) labeled with a radioactive substance or an enzyme, or Protein A, and detecting the monoclonal antibody bound to the solid phase; a method which comprises adding the supernatant 20 of hybridoma to a solid phase adsorbed with an anti-immunoglobulin antibody or Protein A, adding the protein of the present invention labeled with a radioactive substance or an enzyme and detecting the monoclonal antibody bound to the solid phase; and the 25 like.

The monoclonal antibody can be selected according 30 to publicly known methods or their modifications. In general, the selection can be effected in a medium for animal cells supplemented with HAT (hypoxanthine, aminopterin and thymidine). Any selection and growth medium can be employed as far as the hybridoma can grow there. For example, RPMI 1640 medium containing 1% to 35 20%, preferably 10% to 20% fetal bovine serum, GIT medium (Wako Pure Chemical Industries, Ltd.) containing 1% to 10% fetal bovine serum, a serum free medium for

PROPOSED PRACTICE

hybridoma cultivation (SFM-101, Nissui Seiyaku Co., Ltd.) and the like can be used. The cultivation is carried out generally at 20°C to 40°C, preferably at about 37°C, for about 5 days to about 3 weeks, 5 preferably 1 to 2 weeks, normally in 5% CO₂. The antibody titer of the culture supernatant of a hybridoma can be determined as in the assay for the antibody titer in antisera described above.

10 (b) Purification of monoclonal antibody
Separation and purification of a monoclonal antibody can be carried out by publicly known methods, such as separation and purification of immunoglobulins (for example, salting-out, alcohol precipitation, isoelectric point precipitation, electrophoresis, 15 adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method which comprises collecting only an antibody with an activated adsorbent such as an antigen-binding solid phase, Protein A, Protein G, etc., 20 and dissociating the binding to obtain the antibody.

25 [Preparation of polyclonal antibody]

The polyclonal antibody of the present invention can be manufactured by publicly known methods or modifications thereof. For example, a mammal is immunized with an immunogen (the protein antigen of the present invention) per se, or a complex of immunogen and a carrier protein is formed, and a mammal is 25 immunized with the complex in a manner similar to the method described above for the manufacture of monoclonal antibodies. The product containing the antibody to the protein of the present invention is collected from the immunized animal followed by separation and purification of the antibody.

30 In the complex of immunogen and carrier protein 35 used to immunize a mammal, the type of carrier protein

and the mixing ratio of a carrier to a hapten may be any type and in any ratio, as long as the antibody is efficiently produced to the hapten immunized by crosslinking to the carrier. For example, bovine serum 5 albumin, bovine thyroglobulin or keyhole limpet hemocyanin is coupled to one hapten in a carrier-to-hapten weight ratio of approximately 0.1 to 20, preferably about 1 to about 5.

A variety of condensation agents can be used for 10 the coupling of carrier to hapten. Glutaraldehyde, carbodiimide, maleimide activated esters and activated ester reagents containing thiol group or dithiopyridyl group are used for the coupling.

The condensation product is administered to warm- 15 blooded animals either solely or together with carriers or diluents to the site that can produce the antibody by the administration. In order to potentiate the antibody productivity upon administration, complete Freund's adjuvant or incomplete Freund's adjuvant may 20 be administered. The administration is usually made once approximately every 2 to 6 weeks and about 3 to about 10 times in total.

The polyclonal antibody can be collected from the 25 blood, ascites, etc., preferably from the blood of mammal immunized by the method described above.

The polyclonal antibody titer in antiserum can be assayed by the same procedure as that for the determination of serum antibody titer described above. The separation and purification of the polyclonal 30 antibody can be carried out, following the method for the separation and purification of immunoglobulins performed as in the separation and purification of monoclonal antibodies described hereinabove.

The protein of the present invention, the partial 35 peptide, its amides or esters, or salts thereof and the

DNA encoding the same can be used for preparation of antibodies and antisera; construction of systems for expressing the protein of the present invention; construction of systems for assaying the GABA transporter activity using the expression systems and screening of pharmaceutical candidate compounds; effecting drug design based on the steric structure of GABA transporters; reagents for preparation of probes and PCR primers for gene diagnosis; production of transgenic animals; or pharmaceutical drugs for the gene prophylaxis and gene therapy.

In particular, by using the system assaying the GABA transporter activity using the expression system of the protein of the present invention, a compound 15 that alters the GABA transporter activity specific to human or mammal can be screened, and the compound can be used as an agent for the prevention/treatment of various diseases.

The protein of the present invention, the partial peptide, its amide or ester, or salts thereof (hereinafter sometimes merely referred to as the protein of the present invention), the DNA encoding the protein of the present invention or its partial peptide (hereinafter sometimes merely referred to as the DNA of the present invention) and the cells that express the protein of the present invention and the antibody to the protein of the present invention (hereinafter sometimes merely referred to as the antibody of the present invention) are specifically explained below, with respect to their applications.

(1) Method of screening the compound that alters the activities of the protein of the present invention

Using the protein of the present invention, or by applying the GABA transporter activity assay system 35 using the constructed expression system of the protein

of the present invention, the compound (e.g., a peptide, protein, non-peptide compound, synthetic compound, fermentation product, etc.) or its salt that alters the GABA transporter activity of the protein in accordance 5 with the present invention can be efficiently screened.

Such compounds include compounds that potentiate the GABA transporter activity of the protein of the present invention, compounds that inhibit the GABA transporter activity of the protein of the present 10 invention, and the like.

That is, the present invention provides a method of screening a compound or its salt that alters the function (specifically, a GABA transporter activity) of the protein of the present invention, which comprises 15 comparing the case wherein a test compound is contacted with the protein of the present invention, and the case wherein the test compound is not contacted with the protein of the present invention.

More specifically, the present invention provides: 20 a method of screening which comprises comparing the case wherein a test compound is contacted with the protein of the present invention, and the case wherein the test compound is not contacted with the protein of the present invention;

25 a method of screening a compound or its salt that alters the function (specifically, a GABA transporter activity) of the protein of the present invention, which comprises comparing the case wherein a test compound is contacted with a cell containing the 30 protein of the present invention, and the case wherein the test compound is not contacted with the cell containing the protein of the present invention; and,

35 a method of screening a compound or its salt that alters the function (specifically, a GABA transporter activity) of the protein of the present invention,

which comprises comparing the case wherein a test compound is contacted with the protein of the present invention expressed on a cell membrane of a transformant by incubation of the transformant, and the 5 case wherein the test compound is not contacted with the protein of the present invention expressed on the cell membrane of the transformant by incubation of the transformant.

Before the proteins of the present invention were 10 obtained, for screening a compound that alters, e.g., the GABA transporter activity, first, candidate compounds were obtained using cells or tissues containing a GABA transporter protein from rats or other animals (primary screening), and then, the 15 candidate compounds needed to be examined whether the compounds actually alter the GABA transporter activity (secondary screening). When cells or tissues were directly used, other transporter proteins were also intermingled, and it was practically difficult to 20 screen such a compound to alter the objective GABA transporter activity.

However, for example, using the cell capable of expressing the human-derived protein of the present invention, the primary screening becomes unnecessary, 25 and the compound that alters the GABA transporter activity can be efficiently screened.

The screening method of the present invention is specifically described below.

First, the protein of the present invention used 30 for the screening method of the present invention may be any material so long as it contains the protein of the present invention described above. Preferred are cells containing the protein of the present invention derived from mammalian organs. Since it is very 35 difficult to obtain human-derived organs in particular,

however, cells in which the human-derived protein is expressed are suitable for use in the screening.

The cells in which the protein of the present invention is expressed can be constructed by the methods described above, preferably by expressing the DNA of the present invention in mammalian or insect cells. The DNA fragments encoding the objective protein portion include, but are not necessarily limited to, complementary DNA. For example, gene fragments or synthetic DNA may also be used. For introducing DNA fragments encoding the protein of the present invention into host animal cells and efficiently expressing these fragments, it is preferred to insert the DNA fragments downstream the polyhedrin promoter of nuclear polyhedrosis virus (NPV), which is a baculovirus having insect as a host, an SV40-derived promoter, a retrovirus promoter, a metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, an SRα promoter or the like. Any cell is usable for expression as long as the protein of the present invention can be surely expressed in the cells, but it is preferred to use cells having a low GABA transporter activity. The amount and quality of the GABA transporter expressed can be determined by publicly known methods. For example, the determination can be made in accordance with the method for assaying the uptake activity of labeled GABA into the cells (The Journal of Biological Chemistry, 267 (29), 21098-21104 (1992)) or by the method for assaying the activity of binding a compound for inhibiting labeled GABA uptake to the cells (Brain Research, 647, 231-241 (1994)).

More specifically, the cells containing the protein of the present invention are first cultured on a multi-well plate, etc. After the medium is replaced with a buffer suitable for uptake of GABA, a test

compound and labeled GABA are added, followed by incubation for a given period of time and then washing with a buffer suitable for uptake of GABA. Subsequently, the amount of labeled GABA uptake into the cells is 5 determined so that screening can be effected.

Examples of test compounds include a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, a cell extract, a plant extract, an animal tissue extract and the like. These compounds 10 may be novel compounds or publicly known compounds.

The compounds that inhibit the activity of the protein of the present invention are useful as safe and low-toxic medicaments for, e.g., anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, 15 cerebral palsy, spastic spinal paralysis, spondylosis deformans, spinocerebellar degeneration, spastic paralysis accompanied by multiple sclerosis, tension headache, lumbago, neck, shoulder and arm syndrome, etc.

On the other hand, the compounds that accelerate 20 the activity of the protein of the present invention are useful as safe and low-toxic medicaments for, e.g., dementia including Alzheimer disease.

Since the protein of the present invention also has an osmotic pressure regulating action, the 25 compounds that accelerate or inhibit the activity of the protein of the present invention can thus be used as a diuretic in renal ureterolithiasis, as a medicament for the prevention/treatment of intracranial hypotension in brain abscess, ocular hypotension in 30 glaucoma and cerebral edema, as a regulator of cerebral pressure and ocular hypertensive conditions, as a medicament for the treatment of oliguria, edema intracranial hypertension and intracranial edema, an agent for post-treatment after brain surgery, and as a

medicament for the prevention/treatment of oliguria or anuria in acute renal insufficiency.

The screening kit of the present invention comprises the protein of the present invention

5 Protein-expressing cell (specifically, LM (TK⁺ cell, etc.) used in EXAMPLE 5 later described).

Examples of the screening kit according to the present invention include the following:

[Reagents for screening]

10 (1) Buffer for assay

A buffer for GABA uptake (150 mM NaCl, 20 mM HEPES, 1 mM CaCl₂, 10 mM glucose, 5 mM KCl, 1 mM MgCl₂, pH was adjusted to 7.4)

(2) [³H]-GABA

15 (3) Protein preparation of the present invention

When the compound or salts thereof obtainable by the screening method or the screening kit of the present invention are used as the pharmaceutical compositions (agents for the prevention/treatment)

20 described above, the pharmaceutical compositions may be prepared in a conventional manner. For example, the compound or its salts may be prepared into tablets, capsules, elixirs, microcapsules, sterile solutions, suspensions, etc.

25 Since the thus obtained preparations are safe and low toxic, the preparations can be administered to human or mammal (e.g., rat, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.) orally or parenterally.

The dose of the compound or its salt varies
30 depending on, target disease, subject to be administered, route for administration, etc.; when an inhibitor to the protein of the present invention is orally administered for the treatment of, e.g., anxiety, epilepsy, schizophrenia, etc., the inhibitor is
35 normally administered in a dose of about 0.1 mg to

about 100 mg, preferably about 1.0 to about 50 mg, more preferably about 1.0 to about 20 mg per day for adult (as 60 kg body weight). In parenteral administration, the single dose of the compound varies depending on 5 subject to be administered, target disease, etc. but for the purpose of treating, e.g., anxiety, epilepsy, schizophrenia, etc., it is advantageous to administer the inhibitor in the form of injection intravenously at a daily dose of about 0.01 to about 30 mg, preferably 10 about 0.1 to about 20 mg, more preferably about 0.1 to about 10 mg for adult (as 60 kg body weight). For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

15 (1a) Method of screening the compound that alters the GABA transporter activity

Using the protein of the present invention, or by applying the GABA transporter activity assay system using the constructed expression system of the protein of the present invention together with the GABA 20 transporter activity assay system suitably using the other proteins having a GABA transporter activity (GAT-1, GAT-3, BGT-1, etc.), the compound (e.g., a peptide, protein, non-peptide compound, synthetic compound, fermentation product, etc.) or its salt that alters the 25 GABA transporter activity specific to a particular subtype of the GABA transporter can be efficiently screened by comparing the respective activities.

That is, the present invention provides a method of screening a compound or its salt that promotes or 30 inhibits the GABA transporter activity, which comprises assaying the GABA transporter activity using the protein of the present invention.

Such compounds include the compounds that potentiate the GABA transporter activity of the protein 35 of the present invention but do not potentiate (or do

not inhibit) the GABA transporter activity of other subtypes (GAT-1, GAT-3, BTG-1, etc.), the compounds that, the compounds that do not potentiate (or do not inhibit) the GABA transporter activity of the protein 5 of the present invention but potentiate the GABA transporter activity of other subtypes (GAT-1, GAT-3, BTG-1, etc.), and the like.

It is particularly preferred to use the GABA transporter activity assay system using the expression 10 system of the protein of the present invention in combination with the GABA transporter activity assay system using the expression system of GAT-1 or GAT-3 protein.

The GABA transporter activity assay system using 15 other subtypes (GAT-1, GAT-3, BTG-1, etc.) can be constructed as in the GABA transporter activity assay system using the expression system of the protein of the present invention.

The thus obtained compounds that inhibit the GABA 20 transporter activity are useful as safe and low-toxic medicaments for, e.g., anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic spinal paralysis, spondylosis deformans, spinocerebellar degeneration, spastic paralysis 25 accompanied by multiple sclerosis, tension headache, lumbago, neck, shoulder and arm syndrome, etc.

On the other hand, the compounds that accelerate the GABA transporter activity are useful as safe and low-toxic medicaments for, e.g., dementia including 30 Alzheimer disease.

Since the protein of the present invention also has an osmotic pressure regulating action, the compounds that accelerate or inhibit the GABA transporter activity can therefore be used as a 35 diuretic in renal ureterolithiasis, as a medicament for

the prevention/treatment of intracranial hypotension in brain abscess, ocular hypotension in glaucoma and cerebral edema, as a regulator of cerebral pressure and ocular hypertensive conditions, as a medicament for
5 the treatment of oliguria, edema intracranial hypertension and intracranial edema, an agent for post-treatment after brain surgery, and as a medicament for the prevention/treatment of oliguria or anuria in acute renal insufficiency.

10 The screening kit of the present invention comprises the protein of the present invention (preferably, the cell capable of expressing the protein of the present invention and other subtypes (GAT-1, GAT-3, BGT-1, etc.) proteins (preferably, other
15 subtypes (GAT-1, GAT-3, BGT-1, etc.) protein-expressing cells (specifically, LM (TK cell, etc.) used in EXAMPLE 5 later described).

Examples of the screening kit according to the present invention include the following:

20 [Reagents for screening]

(1) Buffer for assay

A buffer for GABA uptake (150 mM NaCl, 20 mM HEPES, 1 mM CaCl₂, 10 mM glucose, 5 mM KCl, 1 mM MgCl₂, pH was adjusted to 7.4)

25 (2) [³H]-GABA

(3) Protein preparation of the invention and other subtypes (GAT-1, GAT-3, BGT-1, etc.) protein preparations

Cells capable of expressing the protein of the present invention and other subtypes (GAT-1, GAT-3, BGT-1, etc.) proteins

When the compound or its salts obtainable by the screening method or the screening kit of the present invention are used as the pharmaceutical compositions described above, the compositions may be prepared in a

conventional manner. For example, the compound or its salts may be prepared into tablets, capsules, elixirs, microcapsules, sterile solutions, suspensions, etc.

The thus obtained preparations are safe and low 5 toxic, and can be administered to human or mammal (e.g., rat, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.) orally or parenterally.

The dose of the compound or its salt varies depending on, target disease, subject to be 10 administered, route for administration, etc.; when an inhibitor to the protein of the present invention is orally administered for the treatment of, e.g., anxiety, epilepsy, schizophrenia, etc., the inhibitor is normally administered in a dose of about 0.1 mg to 15 about 100 mg, preferably about 1.0 to about 50 mg, more preferably about 1.0 to about 20 mg per day for adult (as 60 kg body weight). In parenteral administration, the single dose of the compound varies depending on subject to be administered, target disease, etc. but 20 for the purpose of treating, e.g., anxiety, epilepsy, schizophrenia, etc., it is advantageous to administer the inhibitor in the form of injection intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, more preferably about 0.1 to 25 about 10 mg for adult (as 60 kg body weight). For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

(2) Quantification for the protein of the present 30 invention, its partial peptide, or salts thereof

The antibody of the present is capable of specifically recognizing the protein of the present invention, and can thus be used for quantification of the protein of the present invention in a test sample

fluid, in particular, for quantification by sandwich immunoassay. That is, the present invention provides:

5 (i) a method for quantification of the protein of the present invention or the receptor protein of the present invention in a test sample fluid, which comprises competitively reacting the antibody of the present invention, a test sample fluid and a labeled form of the protein of the present invention, and measuring the ratio of the labeled protein of the

10 present invention bound to said antibody; and,

15 (ii) a method for quantification of the protein of the present invention in a test sample fluid, which comprises reacting a test sample fluid simultaneously or continuously with the antibody of the present invention immobilized on a carrier and a labeled form of the antibody of the present invention, and then assaying the activity of the labeling agent on the insoluble carrier.

20 In the method (ii) described above, it is preferred that one antibody is capable of recognizing the N-terminal region of the protein of the present invention, while another antibody is capable of recognizing the C-terminal region of the protein of the present invention.

25 The monoclonal antibody to the protein of the present invention (hereinafter sometimes referred to as the monoclonal antibody of the present invention) may be used to assay the protein of the present invention. Moreover, the protein of the present invention can be detected by means of a tissue staining as well. For these purposes, the antibody molecule per se may be used, or $F(ab')_2$, Fab' or Fab fractions of the antibody molecule may also be used. Any particular limitation should not be posed on the assay method using the

30 antibody to the protein of the present invention; any

method may be used so far as it is based on a method in which the amount of antibody, antigen or antibody-antigen complex can be detected by a chemical or a physical means, depending on or corresponding to the 5 amount of antigen (e.g., the amount of the protein of the present invention) in a test sample fluid to be assayed, and then calculated using a standard curve prepared by a standard solution containing the known amount of antigen. Advantageously used are, for example, 10 nephrometry, competitive method, immunometric method and sandwich method; in terms of sensitivity and specificity, the sandwich method, which will be described later, is particularly preferred.

Examples of labels used in the assay method using 15 labeling substances are radioisotopes, enzymes, fluorescent substances, luminescent substances, and the like. Examples of the radioisotope are [¹²⁵I], [¹³¹I], [³H], [¹⁴C], etc. Preferred examples of the enzymes are those that are stable and have a high specific activity, 20 which include β -galactosidase, β -glucosidase, alkaline phosphatase, peroxidase and malate dehydrogenase. Examples of the fluorescent substances are fluorescamine, fluorescein isothiocyanate, etc. Examples of the luminescent substances are luminol, 25 luminol derivatives, luciferin, lucigenin, etc. Furthermore, the biotin-avidin system may also be used for binding of an antibody or antigen to a labeling agent.

In the immobilization of antigens or antibodies, 30 physical adsorption may be used. Alternatively, chemical binding that is conventionally used for immobilization of proteins or enzymes may be used as well. Examples of the carrier include insoluble polysaccharides such as agarose, dextran, cellulose,

etc.; synthetic resins such as polystyrene, polyacrylamide, silicone, etc.; glass; etc.

In the sandwich method, a test sample fluid is reacted with an immobilized monoclonal antibody of the present invention (first reaction), then reacted with another labeled monoclonal antibody of the present invention (second reaction) and the activity of the labeling agent on the insoluble carrier is assayed, whereby the amount of the protein of the present invention in the test sample fluid can be quantified. The first and second reactions may be carried out in a reversed order, simultaneously or sequentially with an interval. The type of the labeling agent and the method for immobilization may be performed in a manner similar to those described hereinabove. In the immunoassay by the sandwich method, it is not always necessary that the antibody used for the labeled antibody and for the solid phase should be one type or one species but a mixture of two or more antibodies may also be used for the purpose of improving the measurement sensitivity, etc.

In the method for assaying the protein of the present invention by the sandwich method according to the present invention, antibodies, which binding sites to the protein of the present invention are different from one another, are preferably employed as the monoclonal antibodies of the present invention used for the first and the second reactions. Thus, the antibodies used in the first and the second reactions are those wherein, when the antibody used in the second reaction recognizes the C-terminal region of the protein of the present invention or the receptor protein, the antibody recognizing the site other than the C-terminal regions, e.g., recognizing the N-

terminal region, is preferably used in the first reaction.

The monoclonal antibody of the present invention may be used in an assay system other than the sandwich 5 method, such as a competitive method, an immunometric method and a nephrometry. In the competitive method, an antigen in a test sample fluid and a labeled antigen are competitively reacted with an antibody, then the unreacted labeled antigen (F) and the labeled antigen 10 bound to the antibody (B) are separated (B/F separation) and the labeled amount of either B or F is measured to determine the amount of the antigen in the test sample fluid. In the reactions for such a method, there are a liquid phase method in which a soluble 15 antibody is used as the antibody and the B/F separation is effected by polyethylene glycol while a second antibody to the antibody is used, and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the 20 first antibody while an immobilized antibody is used as the second antibody.

In the immunometric method, an antigen in a test sample fluid and an immobilized antigen are 25 competitively reacted with a given amount of a labeled antibody followed by separating the solid phase from the liquid phase; or an antigen in a test sample fluid and an excess amount of labeled antibody are reacted, then an immobilized antigen is added to bind an unreacted labeled antibody to the solid phase and the 30 solid phase is separated from the liquid phase. Thereafter, the labeled amount of any of the phases is measured to determine the antigen amount in the test sample fluid.

In the nephrometry, the amount of insoluble 35 sediment, which is produced as a result of the antigen-

antibody reaction in a gel or in a solution, is measured. Even when the amount of an antigen in a test sample fluid is small and only a small amount of the sediment is obtained, a laser nephrometry utilizing 5 laser scattering can be suitably used.

In applying each of those immunoassays to the assay method for the present invention, any special conditions or operations are not required to set forth. The assay system for the protein of the present 10 invention may be constructed in addition to conditions or operations conventionally used for each of the methods, taking the technical consideration of one skilled in the art into account consideration. For the details of such conventional technical means, a variety 15 of reviews, reference books, etc. may be referred to (for example, Hiroshi Irie (ed.): "Radioimmunoassay" (published by Kodansha, 1974); Hiroshi Irie (ed.): "Radioimmunoassay; Second Series" (published by Kodansha, 1979); Eiji Ishikawa, et al. (ed.): "Enzyme 20 Immunoassay" (published by Igaku Shoin, 1978); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (Second Edition) (published by Igaku Shoin, 1982); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (Third Edition) (published by Igaku Shoin, 1987); "Methods in 25 Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); ibid., Vol. 73 (Immunochemical Techniques (Part B)); ibid., Vol. 74 (Immunochemical Techniques (Part C)); ibid., Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid., Vol. 92 (Immunochemical 30 Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); ibid., Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (published by Academic Press); etc.)

As described above, the protein of the present invention can be quantified with high sensitivity, using the antibody of the present invention.

(3) Neutralizing antibody

5 In the antibody of the present invention, the neutralizing antibody that can bind to the extracellular region of the protein of the present invention and suppress the function (e.g., the GABA transporter activity) of the protein of the present
10 invention may be used as therapeutic/prophylactic agents for anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic spinal paralysis, spondylosis deformans, spinocerebellar degeneration, spastic paralysis
15 accompanied by multiple sclerosis, tension headache, lumbago, neck, shoulder and arm syndrome, etc. The antibody of the present invention can be prepared into a pharmaceutical composition solely or together with physiologically acceptable carriers such as an aid for
20 promote its uptake, and the composition may be administered to human or other warm-blooded animal.

(4) Antisense DNA

Antisense DNA that can bind to the DNA of the present invention complementarily and suppress
25 expression of the DNA can suppress the function of the protein or DNA of the present invention, and can thus be used as a therapeutic/prophylactic agent for anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic spinal paralysis,
30 spondylosis deformans, spinocerebellar degeneration, spastic paralysis accompanied by multiple sclerosis, tension headache, lumbago, neck, shoulder and arm syndrome, etc.

When the antisense DNA described above is used as
35 the therapeutic/prophylactic agent above, the antisense

DNA is administered directly, or the antisense DNA is inserted into an appropriate vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc., which may then be administered as it 5 stands, or with a physiologically acceptable carrier to assist its uptake by gene gun or through a catheter such as a catheter with a hydrogel.

As the antisense DNA having a base sequence substantially complementary to the DNA encoding the 10 protein of the present invention or its partial peptide (hereinafter sometimes simply referred to as DNA of the present invention), any antisense DNA is usable as long as it has a base sequence substantially complementary to the DNA of the present invention and has the action 15 capable of suppressing expression of the DNA.

The base sequence substantially complementary to the DNA of the present invention may, for example, be a base sequence having at least about 95% homology, preferably at least about 98% homology, to the full-length base sequence or partial base sequence of the 20 base sequence complementary to the DNA of the present invention (i.e., complementary strand to the DNA of the present invention). Particularly in the entire base sequence of the complementary strand to the DNA of the 25 present invention, an antisense DNA having at least about 95% homology, preferably at least about 98% homology, to the complementary strand of the base sequence which encodes the N-terminal region of the protein or its partial protein of the present invention 30 (e.g., the base sequence around the initiation codon). These antisense DNAs can be synthesized using a publicly known DNA synthesizer, etc.

(5) Preparation of non-human animal bearing the DNA encoding the protein of the present invention

Transgenic non-human mammals capable of expressing the protein of the present invention can be prepared using the DNA of the present invention. Examples of the non-human mammals include mammals (e.g., rats, mice, rabbits, sheep, swine, bovine, cats, dogs, monkeys and the like) (hereinafter referred to as animal). Above all, preferred are mice and rats.

To introduce the DNA of the present invention to a target animal, it is generally advantageous to employ the DNA in a gene construct ligated downstream a promoter capable of expressing the DNA in an animal cell. For example, when the mouse-derived DNA of the present invention is transferred, for example, the gene construct, in which the DNA is ligated downstream various promoters capable of expressing the DNA of the present invention derived from an animal that is highly homologous to the DNA of the present invention, is microinjected to mouse fertilized ova. Thus, the DNA-transferred animal capable of producing a high level of the protein of the present invention can be prepared. Examples of the promoters that can be used are a virus-derived promoter and a ubiquitous expression promoter such as metallothionein.

The transfer of the DNA of the present invention at the fertilized egg cell stage secures the presence of DNA in all germ and somatic cells in the target animal. The presence of the protein of the present invention in the germ cells in the DNA-transferred animal means that all germ and somatic cells contain the protein of the present invention in all progenies of the animal. The progenies of the animal that took over the gene contain the protein of the present invention in all germ and somatic cells.

The transgenic animal to which the DNA of the present invention has been transferred can be subjected

to a mating and a breeding for generations under common breeding circumstance, as the DNA-carrying animal, after confirming that the gene can be stably retained. Moreover, male and female animals having the desired 5 DNA are mated to give a homozygote having the transduced gene in both homologous chromosomes and then the male and female animals are mated so that such breeding for generations that progenies contain the DNA can be performed.

10 The transgenic animal to which the DNA of the present invention has been transferred is useful as the animal for screening of drugs that act on the protein of the present invention, since the protein of the present invention is abundantly expressed.

15 The transgenic animal to which the DNA of the present invention has been transferred can also be used as the cell sources for tissue culture. The protein of the present invention can be analyzed by, for example, direct analysis of the DNA or RNA in tissues of the

20 DNA-transferred mice of the present invention, or by analysis of tissues containing the protein of the present invention expressed from the gene. Cells from tissues containing the protein of the present invention are cultured by the standard tissue culture technique.

25 Using these cells, the function of the cells from tissues that are generally difficult to culture, for example, cells derived from the brain and peripheral tissues can be studied. Using these cells it is also possible to screen pharmaceuticals, for example, that 30 increase the function of various tissues. Where a highly expressing cell line is available, the protein of the present invention can be isolated and purified from the cell line.

35 In the specification and drawings, the codes of bases and amino acids are denoted in accordance with

the IUPAC-IUB Commission on Biochemical Nomenclature or by the common codes in the art, examples of which are shown below. For amino acids that may have the optical isomer, L form is presented unless otherwise indicated.

5	DNA : deoxyribonucleic acid
	cDNA : complementary deoxyribonucleic acid
	A : adenine
	T : thymine
	G : guanine
10	C : cytosine
	RNA : ribonucleic acid
	mRNA : messenger ribonucleic acid
	dATP : deoxyadenosine triphosphate
	dTTP : deoxythymidine triphosphate
15	dGTP : deoxyguanosine triphosphate
	dCTP : deoxycytidine triphosphate
	ATP : adenosine triphosphate
	EDTA : ethylenediaminetetraacetic acid
	SDS : sodium dodecyl sulfate
20	Gly : glycine
	Ala : alanine
	Val : valine
	Leu : leucine
	Ile : isoleucine
25	Ser : serine
	Thr : threonine
	Cys : cysteine
	Met : methionine
	Glu : glutamic acid
30	Asp : aspartic acid
	Lys : lysine
	Arg : arginine
	His : histidine
	Phe : phenylalanine
35	Tyr : tyrosine

Trp : tryptophan
 Pro : proline
 Asn : asparagine
 Gln : glutamine
 5 pGlu : pyroglutamic acid
 Me : methyl group
 Et : ethyl group
 Bu : butyl group
 Ph : phenyl group
 10 TC : thiazolidine-4(R)-carboxamide group
 Substituents, protecting groups, and reagents
 frequently used in the specification are denoted by the
 codes below.

15 Tos : p-toluenesulfonyl
 CHO : formyl
 Bzl : benzyl
 Cl₂Bzl : 2,6-dichlorobenzyl
 Bom : benzyloxymethyl
 Z : benzyloxycarbonyl
 20 Cl-Z : 2-chlorobenzyloxycarbonyl
 Br-Z : 2-bromobenzyloxycarbonyl
 Boc : t-butoxycarbonyl
 DNP : dinitrophenol
 Trt : trityl
 25 Bum : t-butoxymethyl
 Fmoc : N-9-fluorenylmethoxycarbonyl
 HOEt : 1-hydroxybenztriazole
 HOOEt : 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-
 benzotriazine
 30 HONB : 1-hydroxy-5-norbornene-2,3-dicarboximide
 DCC : N,N'-dichlorohexylcarbodiimide

The sequence identification numbers (SEQ ID NO:) in
 the sequence listing of the specification indicates the
 35 following sequence, respectively.

[SEQ ID NO: 1]

The sequence shows the amino acid sequence of the protein of the present invention encoded by human GAT2 gene, obtained by EXAMPLE 3 below.

5 [SEQ ID NO: 2]

The sequence shows the base sequence encoded by human GAT2 gene, obtained by EXAMPLE 3 below.

[SEQ ID NO: 3]

10 The sequence shows the base sequence of primer HGA2U used in EXAMPLE 1 below.

[SEQ ID NO: 4]

The sequence shows the base sequence of primer HGA2L used in EXAMPLE 1 below.

[SEQ ID NO: 5]

15 The sequence shows the base sequence of primer HGA2-198L used in EXAMPLE 2 below.

[SEQ ID NO: 6]

The sequence shows the base sequence of primer HGA2-247L used in EXAMPLE 2 below.

20 [SEQ ID NO: 7]

The sequence shows the base sequence of primer HGA2-1665U used in EXAMPLE 2 below.

[SEQ ID NO: 8]

25 The sequence shows the base sequence of primer HGA2-1613U used in EXAMPLE 2 below.

[SEQ ID NO: 9]

The sequence shows the base sequence of primer AP-1 used in EXAMPLE 2 below.

[SEQ ID NO: 10]

30 The sequence shows the base sequence of primer AP-2 used in EXAMPLE 2 below.

[SEQ ID NO: 11]

The sequence shows the base sequence of primer HGA2-U2 used in EXAMPLE 3 below.

35 [SEQ ID NO: 12]

The sequence shows the base sequence of primer HGA2-L2 used in EXAMPLE 3 below.

Transformant *Escherichia coli* DH5 α /pMCMV-hGAT2
5 bearing the plasmid pMCMV-hGAT2 obtained in EXAMPLE 4
later described was on deposit with the Ministry of
International Trade and Industry, Agency of Industrial
Science and Technology, National Institute of
Bioscience and Human Technology (NIBH) at 1-1-3,
10 Higashi, Tsukuba-shi, Ibaraki, Japan, as the Accession
Number FERM BP-6739 on June 2, 1999 and with Institute
for Fermentation, Osaka (IFO) at 2-17-85, Juso Honcho,
Osaka-shi, Osaka, Japan, as the Accession Number IFO
16286 on April 28, 1999.

15

EXAMPLES

Genetic manipulation described in the following
EXAMPLES was performed in accordance with methods
described in the textbook (Maniatis et al., Molecular
20 Cloning, Cold Spring Harbor Laboratory, 1989) or by the
procedures described in the protocols attached to
reagents.

EXAMPLE 1 Partial cloning of human GAT2 gene

25 Using as a template human retina-derived cDNA
(Toyobo, QUICK-Clone cDNA), partial cloning of human
GAT2 gene was carried out by PCR using the primer set
below prepared with reference to the base sequence of
rat GAT2 gene reported by Borden et al. (J. Biol. Chem.,
30 267, 21098-21104 (1992)).

HGA2U: 5'-GGT GGG ATG GAT AAC AGC AGG GTC TCG GGA ACG [SEQ
ID NO:3]

35 HGA2L: 5'-CCC TAG CAG TTA GAC TCC AGT TCT GTG AGC [SEQ
ID NO:4]

The PCR procedure was performed by a Hot Start method using AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.). First, 2 ml of 10 x LA PCR Buffer, 3 ml of 2.5 mM dNTP solution, 2 ml of 25 mM MgCl₂, 2.5 ml each of primer solutions ([SEQ ID NO:3] and [SEQ ID NO:4]) and 8 ml of sterilized distilled water were mixed to obtain a bottom layer solution mixture. One milliliter of human retina cDNA (1 ng/ml) as a template, 3 ml of 10 x LA PCR Buffer, 1 ml of 2.5 mM dNTP solution, 3 ml of 25 mM MgCl₂, 0.5 ml of TaKaRa LA Taq DNA polymerase (TAKARA SHUZO CO., LTD.) and 21.5 ml of sterilized distilled water were mixed to obtain a top layer solution mixture. The bottom solution mixture thus prepared received one unit of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the reaction mixture of PCR. A tube containing the reaction mixture was set on a thermal cycler (Perkin Elmer) and treated at 95°C for 2 minutes. After repeating the cycle of 98°C for 10 seconds followed by 60°C for 30 seconds and then 72°C for 2 minutes 45 times, the tube was further treated at 72°C for 8 minutes. The PCR product thus obtained was subjected to electrophoresis on agarose gel (1%) to recover 1.8 kb DNA fragment containing GAT2 gene from the gel. Then, the fragment was inserted into pT7 Blue vector (TAKARA SHUZO CO., LTD.) to obtain a plasmid designated as pT7-GAT2.

Confirmation of the base sequence for the PCR fragment part of pT7-GAT2 shows that the base sequence contained a sequence similar to rat-derived GAT2 gene.

EXAMPLE 2

Cloning of human GAT2 gene at the 5' and 3' regions

The 5' and 3' regions in the sequence of the PCR fragment obtained in EXAMPLE 1 are sequences of rat GAT2 gene. Thus, for cloning human GAT2 gene at the 5' and 3' regions, the cloning was carried out using as 5 templates human retina- and kidney- derived cDNAs (Toyobo, Marathon Ready cDNA), by nested PCR procedures of a RACE (Rapid Amplification of cDNA End) method using the following specific primer set prepared with reference to the base sequence of pT7-GAT2 gene:

10

HGA2-198L:

5'-GCA CCT CCC CCA TTT TTG TAG CAG [SEQ ID NO:5]

HGA2-247L:

5'-GAC AGG AAT GCC ACA GGT AAA GAG [SEQ ID NO:6]

15 HGA2-1665U:

5'-CTC TAC AGA CTC GGA ACC CTC AAG [SEQ ID NO:7]

HGA2-1613U:

5'-CCT GGG CTG GCT CCT GGC TCT GTC [SEQ ID NO:8]

and the primer set attached to Marathon Ready cDNA:

20 AP-1:

5'-CCA TCC TAA TAC GAC TCA CTA TAG GGC [SEQ ID NO:9]

AP-2:

5'-ACT CAC TAT AGG GCT CGA GCG GC [SEQ ID NO:10]

25

The first PCR procedure for the 5' region was performed by a Hot Start method using AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.). A bottom layer solution 30 mixture was prepared by mixing 2 ml of 10 x pyrobest DNA polymerase Buffer, 3 ml of 2.5 mM dNTP solution, 1 ml each of primer solutions (HGA2-247L [SEQ ID NO:6] and AP-1 [SEQ ID NO:9]) and 13 ml of sterilized distilled water. Five milliliters of human retina- and 35 kidney-derived cDNAs as a template, 3 ml of 10 x

pyrobest DNA polymerase Buffer, 1 ml of 2.5 mM dNTP solution, 0.5 ml of pyrobest DNA polymerase (TAKARA SHUZO CO., LTD.) and 20.5 ml of sterilized distilled water were mixed to obtain a top layer solution mixture.

5 The bottom solution mixture thus prepared received one unit of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the reaction mixture of 10 PCR. A tube charged with the reaction mixture was set on a thermal cycler (Perkin Elmer) and treated at 94°C for 2 minutes. The cycle of 94°C for 5 seconds and 72°C for 3 minutes was repeated 5 times, then the cycle of 94°C for 5 seconds and 70°C for 3 minutes 5 times, 15 followed by the cycle of 94°C for 5 seconds and then 68°C for 3 minutes 25 times. Then, the nested PCR was performed using the PCR product obtained as a template. That is, 2 ml of 10 x LA PCR Buffer, 3 ml of 2.5 mM dNTP solution, 2 ml of 25 mM MgCl₂, 1 ml each of primer 20 solutions (HGA2-198L [SEQ ID NO:5] and AP-2 [SEQ ID NO:10]) and 11 ml of sterilized distilled water were mixed to obtain a bottom layer solution mixture. One milliliter of the PCR products of HGA2-247L and AP-1 at the 5' region as templates, 3 ml of 10 x LA PCR Buffer, 25 1 ml of 2.5 mM dNTP solution, 3 ml of 25 mM MgCl₂, 0.5 ml of TaKaRa LA Taq DNA polymerase (TAKARA SHUZO CO., LTD.) and 21.5 ml of sterilized distilled water were mixed to obtain a top layer solution mixture. The bottom solution mixture thus prepared received one unit 30 of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the reaction mixture of PCR. A tube charged with the reaction mixture was set on a thermal cycler (Perkin Elmer) and treated at 94°C for 2 minutes. 35

The cycle of 94°C for 5 seconds and 72°C for 3 minutes was repeated 5 times, then the cycle of 94°C for 5 seconds and 70°C for 3 minutes 5 times, followed by the cycle of 94°C for 5 seconds and then 68°C for 3 minutes 5 times.

The first PCR procedure for the 3' region was performed by a Hot Start method using AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.). A bottom layer solution mixture was prepared by mixing 2 ml of 10 x pyrobest DNA polymerase Buffer, 3 ml of 2.5 mM dNTP solution, 1 ml each of primer solutions (HGA2-1613U [SEQ ID NO:8] and AP-1 [SEQ ID NO:9]) and 13 ml of sterilized distilled water. Five milliliters of human retina- and kidney-derived cDNAs as a template, 3 ml of 10 x pyrobest DNA polymerase Buffer, 1 ml of 2.5 mM dNTP solution, 0.5 ml of pyrobest DNA polymerase (TAKARA SHUZO CO., LTD.) and 20.5 ml of sterilized distilled water were mixed to obtain a top layer solution mixture. The bottom solution mixture thus prepared received one unit of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the reaction mixture of PCR. A tube charged with the reaction mixture was set on a thermal cycler (Perkin Elmer) and treated at 94°C for 2 minutes. The cycle of 94°C for 5 seconds and 72°C for 3 minutes was repeated 5 times, then the cycle of 94°C for 5 seconds and 70°C for 3 minutes 5 times, followed by the cycle of 94°C for 5 seconds and then 68°C for 3 minutes 25 times. Then, the nested PCR was performed using the PCR product obtained as a template. That is, 2 ml of 10 x pyrobest DNA polymerase Buffer, 3 ml of 2.5 mM dNTP solution, 1 ml each of primer solutions (HGA2-1665U [SEQ ID NO:7] and AP-2 [SEQ ID NO:10]) and 13 ml of sterilized distilled water were

mixed to obtain a bottom layer solution mixture. One milliliter of the PCR products of HGA2-1613U and AP-1 at the 3' region as templates, 3 ml of 10 x pyrobest DNA polymerase Buffer, 1 ml of 2.5 mM dNTP solution,

5 0.5 ml of pyrobest DNA polymerase (TAKARA SHUZO CO., LTD.) and 24.5 ml of sterilized distilled water were mixed to obtain a top layer solution mixture. The bottom solution mixture thus prepared received one unit of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and
10 was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the reaction mixture of PCR. A tube charged with the reaction mixture was set on a thermal cycler (Perkin Elmer) and treated at 94°C for 2 minutes.

15 The cycle of 94°C for 5 seconds and 72°C for 3 minutes was repeated 5 times, then the cycle of 94°C for 5 seconds and 70°C for 3 minutes 5 times, followed by the cycle of 94°C for 5 seconds and then 68°C for 3 minutes 25 times.

20 Direct sequencing of the respective PCR products thus obtained shows that the products contained sequences similar to rat-derived GAT2 gene at the 5' and 3' regions.

25 EXAMPLE 3

Cloning of the full-length of human GAT2 gene

Using three different templates of human kidney QUICK-Clone cDNA (Toyobo) and human retina and kidney Marathon Ready cDNAs (Toyobo), cloning of the full-length human GAT2 gene was carried out by PCR using the specific primer set below prepared with reference to the base sequences of 5' and 3' regions obtained in EXAMPLE 2.

35 HGA2-U2:

5'-GGC AGC GCT AGC AGG TCT GGC AGC AGC TTC ACT AAG
[SEQ ID NO:11]

HGA2-L2:

5' -TCA CCA GTC GAC GGC ACA CAG GCA CCA TCC AAG GGC
5 [SEQ ID NO:12]

The PCR procedure was performed by a Hot Start method using AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.). When human kidney QUICK-Clone cDNA (Toyobo) was 10 used as a template, a bottom layer solution mixture was prepared by mixing 2 ml of 10 x pyrobest DNA polymerase Buffer, 3 ml of 2.5 mM dNTP solution, 1 ml each of primer solutions ([SEQ ID NO:11] and [SEQ ID NO:12]) and 13 ml of sterilized distilled water. One milliliter 15 of the template, 3 ml of 10 x pyrobest DNA polymerase Buffer, 1 ml of 2.5 mM dNTP solution, 0.5 ml of pyrobest DNA polymerase (TAKARA SHUZO CO., LTD.) and 24.5 ml of sterilized distilled water were mixed to obtain a top layer solution mixture. The bottom 20 solution mixture thus prepared received one unit of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the reaction mixture of PCR. When 25 human retina- and kidney Marathon Ready cDNAs (Toyobo) were used as templates, a bottom layer solution mixture was prepared by mixing 2 ml of 10 x pyrobest DNA polymerase Buffer, 3 ml of 2.5 mM dNTP solution, 1 ml each of the primer solutions and 13 ml of sterilized 30 distilled water. Five milliliters of the template, 3 ml of 10 x pyrobest DNA polymerase Buffer, 1 ml of 2.5 mM dNTP solution, 0.5 ml of pyrobest DNA polymerase (TAKARA SHUZO CO., LTD.) and 20.5 ml of sterilized distilled water were mixed to obtain a top layer 35 solution mixture. The bottom solution mixture thus

prepared received one unit of AmpliWax PCR Gem 100 (TAKARA SHUZO CO., LTD.), and was treated at 70°C for 5 minutes and then in ice for 5 minutes. Thereafter, the top layer solution mixture was added to prepare the 5 reaction mixture of PCR. A tube charged with the reaction mixture was set on a thermal cycler (Perkin Elmer) and treated at 95°C for 2 minutes. The cycle of 98°C for 10 seconds, 60°C for 30 seconds and 72°C for 2 minutes was repeated 45 times, then the mixture was 10 treated at 72°C for 8 minutes. The respective PCR products thus obtained were subjected to electrophoresis on agarose gel (1%) to recover 1.8 kb DNA fragment containing human GAT2 gene from the gel. Then, the fragment was inserted into pT7 Blue vector 15 (TAKARA SHUZO CO., LTD.) to produce pT7-hGAT2 No. 1-11 derived from human kidney QUICK-Clone cDNA, pT7-hGAT2 No. 3-6 derived from human kidney Marathon Ready cDNA, and pT7-hGAT2 No. 4-13 derived from human retina Marathon Ready cDNA.

20 Since the base sequences for the PCR fragment parts derived from the three different templates all coincided with each other, it was confirmed that human GAT2 gene [SEQ ID NO:2] was acquired.

25 EXAMPLE 4

Preparation of plasmid for human GAT 2 expression

By ligating 5.6 Kb NheI-SalI fragment from plasmid pMCMVneo with 1.8 Kb NheI-SalI fragment bearing human GAT2 gene of plasmid pT7-hGAT2 No. 4-13, plasmid pMCMV-30 hGAT2 was produced.

Using plasmid pMCMV-hGAT2, Escherichia coli DH5 α strain was transfected to acquire Escherichia coli DH5 α /pMCMV-hGAT2.

35 EXAMPLE 5

Introduction of human GAT2-expressing plasmid into LM(TK⁻) cell and acquisition of the expressed cell

A LM(TK⁻) cell cultured in a 750 ml tissue culture flask (Corning) containing DMEM medium (Lifetech

5 Oriental) supplemented with 10% fetal bovine serum (Lifetech Oriental) was scraped by treating with 0.5 g/L trypsin-0.2 g/L EDTA (Lifetech Oriental). The cell was washed with PBS (Lifetech Oriental), centrifuged (1000 rpm, 5 minutes) and then suspended in PBS.

10 Subsequently, a DNA was introduced into the cell under the conditions shown below using GENE PULSER (Bio-Rad Laboratories). That is, a cuvette having a 0.4 cm gap received 8×10^6 cells and 10 mg of plasmid pMCMV-hGAT2 for expression and was then subjected to

15 electroporation at the voltage of 0.25 kV under the capacitance of 960 mF. Subsequently, the cell was transferred to a DMEM medium containing 10% fetal bovine serum and cultured for 24 hours. Thereafter, the cell was scraped again, centrifuged, and then suspended

20 in DMEM medium containing 10% fetal bovine serum supplemented with 500 mg/ml of GENETICIN (Lifetech Oriental) and diluted to a level of 10^4 cells/ml upon inoculation onto a 96-well plate (Becton-Dickinson), followed by incubation in a carbonate gas incubator at

25 37°C. Thus, a GENETICIN-resistant transformant was acquired.

Subsequently, GAT2 expression was confirmed by the following procedures. That is, after the transformant cell line thus obtained was cultured in a 96-well plate (Corning), the medium was replaced by GABA uptake

30 Buffer (150 mM NaCl, 20 mM HEPES, 1 mM CaCl₂, 10 mM Glucose, 5 mM KCl, 1 mM MgCl₂; pH was adjusted to 7.4) and then 50 nM [³H]-GABA was added thereby to screen a cell line in which the [³H]-GABA uptake was induced, 35 i.e., hGAT2/LM(TK⁻) (FIG. 2).

INDUSTRIAL APPLICABILITY

The protein or its salt, the partial protein, its amide or ester, or salts thereof, and DNA encoding the 5 same can be employed for obtaining antibodies and antisera, constructing the expression system of the protein of the present invention, for constructing the assay system for the GABA transporter activity and screening medicament candidate compounds using the 10 expression system, for making drug design based on the steric structure of GABA transporters, as agents for producing probes or PCR primers in gene diagnosis, for preparing transgenic animals or as pharmaceutical compositions for the prevention/treatment of gene, etc.

15

CLAIMS

1. A protein containing an amino acid sequence, which is the same or substantially the same amino acid sequence represented by SEQ ID NO:1, or its salt.

2. The protein or its salt according to claim 1, which has a GABA transporter activity.

3. A partial peptide of the protein according to claim 1, its amide or ester, or a salt thereof.

4. A DNA containing a DNA having the base sequence encoding the protein according to claim 1 or the partial peptide according to claim 3.

5. The DNA according to claim 4 having the base sequence represented by SEQ ID NO:2.

6. A recombinant vector containing the DNA according to claim 4.

7. A transformant transformed with the recombinant vector according to claim 6.

8. A process for producing the protein or its salt, according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, which comprises culturing said transformant of claim 7, producing and accumulating the protein according to claim 1 or the partial peptide according to claim 3, and collecting the protein or partial peptide.

9. An antibody to the protein or its salt according to claim 1 or to the partial peptide, its amide or ester, or a salt thereof according to claim 3.

10. A method of screening a compound that promotes or inhibits a GABA transporter activity, which comprises assaying the GABA transporter activity using the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof according to claim 3.

11. A method of screening a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, which comprises using the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3.

12. A kit for screening a compound or its salt that promotes or inhibits a GABA transporter activity, comprising the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3.

13. A kit for screening a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, comprising the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3.

14. A compound or its salt that promotes or inhibits a GABA transporter activity, obtainable using the screening method according to claim 10 or using the screening kit according to claim 12.

15. A compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, which is obtainable using the screening method according to claim 11 or using the screening kit according to claim 13.

16. A pharmaceutical comprising a compound or its salt that promotes or inhibits a GABA transporter activity, which is obtainable using the screening

method according to claim 10 or using the screening kit according to claim 12.

17. A pharmaceutical comprising a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, which is obtainable using the screening method according to claim 11 or using the screening kit according to claim 13.

18. A pharmaceutical comprising a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, which is a medicament for the prevention/treatment of anxiety, spasm, epilepsy, schizophrenia, cerebrovascular disorders, cerebral palsy, spastic spinal paralysis, spondylosis deformans, spinocerebellar degeneration, spastic paralysis accompanied by multiple sclerosis, tension headache, lumbago, and neck, shoulder and arm syndrome.

19. A pharmaceutical comprising a compound or its salt that promotes or inhibits a GABA transporter activity of the protein or its salt according to claim 1, or the partial peptide, its amide or ester, or a salt thereof, according to claim 3, which is a medicament for the prevention/treatment of dementia.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-208 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Novel Protein And DNA Thereof

which is described and claimed in:

the specification attached hereto.

the specification in U.S. Application Serial Number _____, filed on _____.

the specification in PCT international application Number PCT/JP00/03720, filed on June 8, 2000; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201 Hiroyuki Kimura	Date: <i>Oct 15, 2001</i>
Signature of Inventor 202 Junichi Sakamoto	Date: <i>Oct 15, 2001</i>
Signature of Inventor 203 Hidekazu Sawada	Date: <i>Oct 17, 2001</i>
Signature of Inventor 204	Date:
Signature of Inventor 205	Date:

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INDEXED
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FILED

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S.-Benefit Under 35 U.S.C. §120

U.S. Applications		Status (Check One)		
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. §119(e))**

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

and Trademark Office connected therewith.

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David G. Conlin (Reg. No. <u>27,026</u>)	Cara Z. Lowen (Reg. No. <u>38,227</u>)	David A. Tucker (Reg. No. <u>27,840</u>)
George W. Neuner (Reg. No. <u>26,964</u>)	William J. Daley, Jr. (Reg. No. <u>35,487</u>)	Lisa Hazzard Swiszcz (Reg. No. <u>44,368</u>)
Linda M. Buckley (Reg. No. <u>31,003</u>)	Robert L. Buchanan (Reg. No. <u>40,927</u>)	
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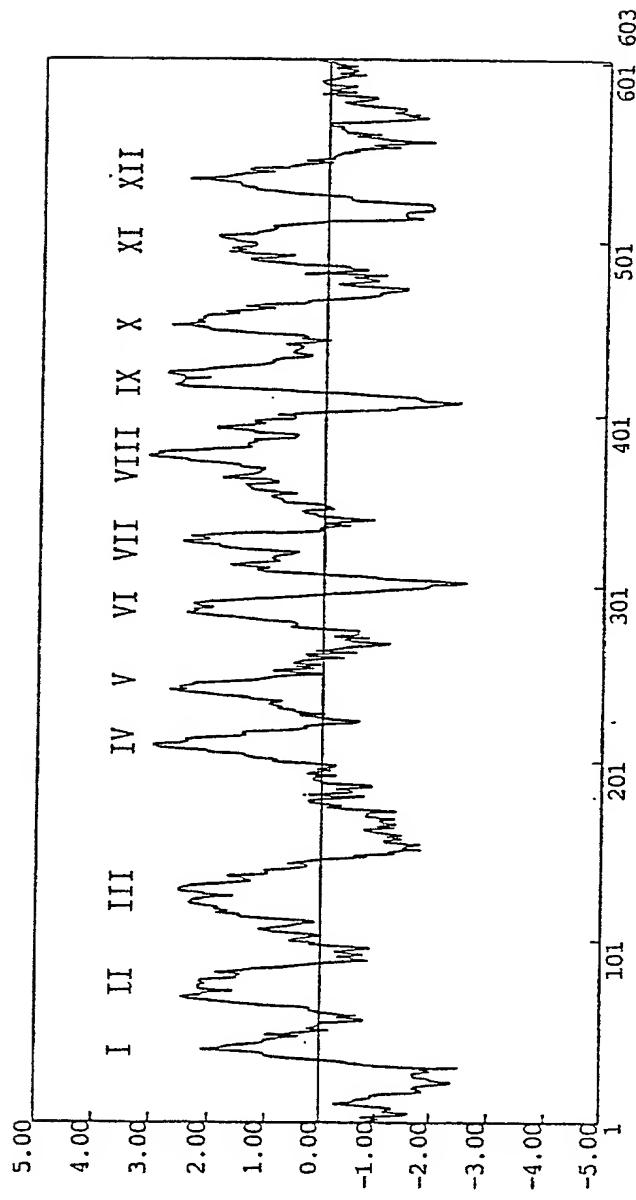
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10/009693

1/2

Fig. 1



10/009693

[Sequence Listing]

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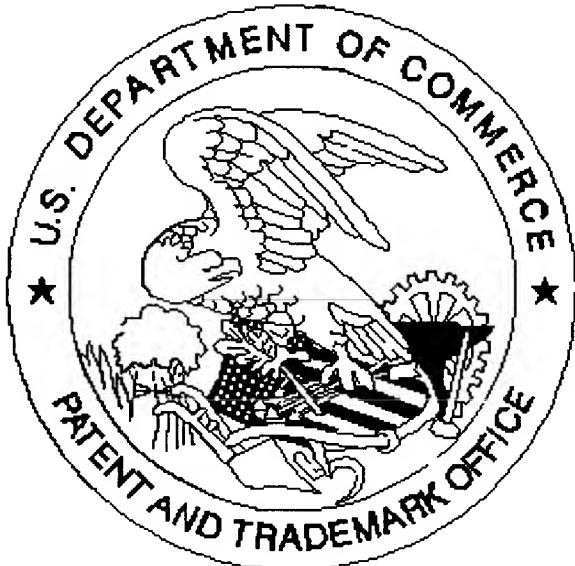
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